

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	41	phospholamban	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/2 1 07:31		0	
2	BRS	L2	0	1 same (three adj dimensional adj structure)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/2 1 07:32		0	
3	BRS	L3	0	1 same (x adj ray adj structure)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/2 1 07:33		0	
4	BRS	L4	0	1 same (molecular adj model\$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2001/12/2 1 07:33		1	Truncation Over flow . Return string from Server is: 5`54 0449`

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=> s phospholamban  
L1 3823 PHOSPHOLAMBAN

=> s l1 (p) (three dimensional structure)  
L2 15 L1 (P) (THREE DIMENSIONAL STRUCTURE)

=> s l1 (p) (x ray structure)  
L3 0 L1 (P) (X RAY STRUCTURE)

=> s l1 (p) (molecular model?)  
L4 31 L1 (P) (MOLECULAR MODEL?)

=> s l2 or l4  
L5 44 L2 OR L4

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PROCESSING COMPLETED FOR L5  
L6 20 DUPLICATE REMOVE L5 (24 DUPLICATES REMOVED)

=> d l6 1-20 ibib abs

L6	ANSWER 1 OF 20	MEDLINE	DUPLICATE 1
ACCESSION NUMBER:	2001553881	IN-PROCESS	
DOCUMENT NUMBER:	21486400	PubMed ID: 11477077	
TITLE:	Role of cysteine residues in structural stability and		

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function of a transmembrane helix bundle.  
AUTHOR: Karim C B; Paterlini M G; Reddy L G; Hunter G W; Barany G; Thomas D D  
CORPORATE SOURCE: Departments of Biochemistry, Molecular Biology, and Biophysics, Medicinal Chemistry, and Chemistry, University of Minnesota, Minneapolis; Minnesota 55455.  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Oct 19) 276 (42) 38814-9.  
Journal code: HIV; 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals  
ENTRY DATE: Entered STN: 20011016  
Last Updated on STN: 20011016

AB To study the structural and functional roles of the cysteine residues at positions 36, 41, and 46 in the transmembrane domain of **phospholamban** (PLB), we have used Fmoc (N-(9-fluorenyl)methoxycarbonyl) solid-phase peptide synthesis to prepare alpha-amino-n-butyric acid (Abu)-PLB, the analogue in which all three cysteine residues are replaced by Abu. Whereas previous studies have shown that replacement of the three Cys residues by Ala (producing Ala-PLB) greatly destabilizes the pentameric structure, we hypothesized that replacement of Cys with Abu, which is isosteric to Cys, might preserve the pentameric stability. Therefore, we compared the oligomeric structure (from SDS-polyacrylamide gel electrophoresis) and function (inhibition of the Ca-ATPase in reconstituted membranes) of Abu-PLB with those of synthetic wild-type PLB and Ala-PLB. **Molecular modeling** provides structural and energetic insight into the different oligomeric stabilities of these molecules. We conclude that 1) the Cys residues of PLB are not necessary for pentamer formation or inhibitory function; 2) the steric properties of cysteine residues in the PLB transmembrane domain contribute substantially to pentameric stability, whereas the polar or chemical properties of the sulfhydryl group play only a minor role; 3) the functional potency of these PLB variants does not correlate with oligomeric stability; and 4) acetylation of the N-terminal methionine has neither a functional nor a structural effect in full-length PLB.

L6 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:34634 CAPLUS  
DOCUMENT NUMBER: 134:218589  
TITLE: Reexamination of the role of the leucine/isoleucine zipper residues of phospholamban in inhibition of the Ca<sup>2+</sup> pump of cardiac sarcoplasmic reticulum  
AUTHOR(S): Cornea, Razvan L.; Autry, Joseph M.; Chen, Zhenhui; Jones, Larry R.  
CORPORATE SOURCE: Department of Medicine and the Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN, 46202, USA  
SOURCE: J. Biol. Chem. (2000), 275(52), 41487-41494  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal

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LANGUAGE: English

AB Phospholamban is a small phosphoprotein inhibitor of the  $\text{Ca}^{2+}$ -pump in cardiac sarcoplasmic reticulum, which shows a distinct oligomeric distribution between monomers and homopentamers that are stabilized through Leu/Ile zipper interactions. A two-faced model of phospholamban inhibition of the  $\text{Ca}^{2+}$ -pump was proposed, in which the Leu/Ile zipper residues located on one face of the transmembrane  $\alpha$ -helix regulate the pentamer to monomer equil., whereas residues on the other face of the helix bind to and inhibit the pump. Here we tested this two-faced model of phospholamban action by analyzing the functional effects of a new series of Leu/Ile zipper mutants. Pentameric stabilities of the mutants were quantified at different SDS concns. We show that several phospholamban mutants with hydrophobic amino acid substitutions at the Leu/Ile zipper region retain the ability to form pentamers but at the

same time give the same or even stronger (i.e. L37I-PLB) inhibition of the  $\text{Ca}^{2+}$ -pump than do mutants that are more completely monomeric. Steric constraints prevent the Leu/Ile zipper residues sequestered in the interior of the phospholamban pentamer from binding to the  $\text{Ca}^{2+}$ -pump, leading to the conclusion that the zipper residues access the pump from the phospholamban monomer, which is the active inhibitory species. A modified model of phospholamban transmembrane domain action is proposed, in which the membrane span of the phospholamban monomer maintains contacts

with the  $\text{Ca}^{2+}$ -pump around most of its circumference, including residues located in the Leu/Ile zipper region.

REFERENCE COUNT: 25

REFERENCE(S): (2) Asahi, M; J Biol Chem 1999, V274, P32855 CAPLUS  
(3) Autry, J; Ann NY Acad Sci 1998, V853, P92 CAPLUS  
(4) Autry, J; J Biol Chem 1997, V272, P15872 CAPLUS  
(5) Cantilina, T; J Biol Chem 1993, V268, P17018 CAPLUS  
(6) Chu, G; J Biol Chem 1998, V273, P33674 CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:758728 CAPLUS

DOCUMENT NUMBER: 134:67735

TITLE: Deuterium NMR Reveals Helix Packing Interactions in Phospholamban

AUTHOR(S): Ying, Weiwen; Irvine, Scott E.; Beekman, Richard A.; Siminovitch, David J.; Smith, Steven O.

CORPORATE SOURCE: Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY, 11794-5215, USA

SOURCE: J. Am. Chem. Soc. (2000), 122(45), 11125-11128  
CODEN: JACSAT; ISSN: 0002-7863

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Phospholamban is an integral membrane protein having a single membrane-spanning helix which forms a pentameric complex in cardiac and smooth muscle cell membranes. Deuterium NMR measurements of leucine residues in the transmembrane domain of the protein provide a novel approach for establishing the rotational orientation of the phospholamban monomer within the complex. At 5.degree., the spectra of Leu43 and Leu44 are similar and exhibit a quadrupole splitting of 33 kHz. This splitting is slightly narrower than the .apprx.40 kHz splitting which results solely

from rapid Me group rotation. The deuterium line shape of Leu42 has lost the distinctive 33-kHz quadrupole splitting due to increased librational motion of the side chain and/or rotation about the C.alpha.-C.beta. and C.beta.-C.gamma. bonds. The obsd. line shapes of the three consecutive leucine residues in phospholamban are consistent with Leu42 being oriented

toward the lipids, where it exhibits fewer steric contacts, and Leu43 and Leu44 being oriented toward helix interfaces which restrict their motion. Possible packing arrangements of the three transmembrane leucine residues in the phospholamban pentamer are examd. using computational methods to assess the packing restrictions of the leucine side chains. The results are discussed in terms of models of the phospholamban pentamer previously proposed on the basis of mutational data.

REFERENCE COUNT: 28  
 REFERENCE(S): (1) Adams, P; Nature Struct Biol 1995, V2, P154  
 CAPLUS (2) Arkin, I; EMBO J 1994, V13, P4757 CAPLUS  
 (3) Arkin, I; J Mol Biol 1995, V248, P824 CAPLUS  
 (4) Batchelder, L; Proc Natl Acad Sci U S A 1982, V79, P386 CAPLUS  
 (5) Beshah, K; J Chem Phys 1987, V86, P4730 CAPLUS  
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 2  
 ACCESSION NUMBER: 2000:748484 CAPLUS  
 DOCUMENT NUMBER: 134:96807  
 TITLE: NMR solution structure of phospholamban  
 AUTHOR(S): Lamberth, Stefanie; Schmid, Holger; Muenchbach, Martin; Vorherr, Thomas; Krebs, Joachim; Carafoli, Ernesto; Griesinger, Christian  
 CORPORATE SOURCE: Institute of Organic Chemistry, University of Frankfurt, Frankfurt, D-60439, Germany  
 SOURCE: Helv. Chim. Acta (2000), 83(9), 2141-2152  
 CODEN: HCACAV; ISSN: 0018-019X  
 PUBLISHER: Verlag Helvetica Chimica Acta  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB **Phospholamban** (PLN), an amphipathic intrinsic membrane protein of 52 amino acids, is the modulator of the Ca<sup>2+</sup> pump of cardiac, slow-twitch, and smooth-muscle sarcoplasmic reticulum. In response to .beta.-adrenergic stimulation, it becomes phosphorylated at Ser16 and/or Thr17, and disassociates from the pump, which, in turn, achieves its activity. Here we present the **three-dimensional structure** of chem. synthesized, monomeric PLN in an org. solvent. Monomerization (PLN normally forms homopentamers) was obtained by replacing Cys41 with phenylalanine (Phe=F), a modification that did not affect biol. activity. The structure was detd. by high-resoln. NMR in CHCl<sub>3</sub>/MeOH of the unphosphorylated state of [F41]PLN (C41F). Of the hydrophilic cytoplasmic parts IA (Met1 to Pro21) and IB (Gln22 to Asn30) and the membrane-spanning hydrophobic domain II (Leu31 to Leu52) of PLN, domain IA, which contains the two phosphorylation sites Ser16 and Thr17, and domain II have been suggested to be helical and connected through the less-structured hinge-region IB. In the structural study presented here, [F41]PLN is composed of two .alpha.-helical regions connected by a .beta.-turn (type III). The residues of the .beta.-turn (type III) are Thr17, Ile18, Glu19, and Met20, the first being one of the two phosphorylation sites (Ser16 and Thr17). The hinge region is located at

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the C-terminal end of domain IA, and domain IB is part of a second helix. The two .alpha.-helices comprising amino acids 4-16 and 21-49 are well-defined (the root-mean-square deviations for the backbone atoms, calcd. for a family of the structures, are 0.58 and 0.92 .ANG., resp.). Pro21 is at the beginning of the C-terminal helix and in the trans conformation.

REFERENCE COUNT: 33  
REFERENCE(S): (2) Aue, W; J Chem Phys 1976, V64, P2229 CAPLUS  
(3) Autry, J; J Biol Chem 1997, V272, P15872 CAPLUS  
(4) Braunschweiler, L; J Magn Reson 1983, V53, P521 CAPLUS  
(6) Chu, G; Circ Res 1997, V81, P485 CAPLUS  
(7) Fletcher, C; J Biomol NMR 1996, V8, P292 CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 20 MEDLINE DUPLICATE 3  
ACCESSION NUMBER: 2000502642 MEDLINE  
DOCUMENT NUMBER: 20503369 PubMed ID: 11051085  
TITLE: A fast method for the quantitative estimation of the distribution of hydrophobic and hydrophilic segments in alpha-helices of membrane proteins.  
AUTHOR: Luzhkov V B; Surkov N F  
CORPORATE SOURCE: Institute of Chemical Physics Problems in Chernogolovka, Russian Academy of Sciences, Chernogolovka, Moscow  
Region.. luzhkov@icp.ac.ru  
SOURCE: MEMBRANE AND CELL BIOLOGY, (2000) 14 (1) 89-96.  
Journal code: CWK; 9517472. ISSN: 1023-6597.  
PUB. COUNTRY: Switzerland  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200103  
ENTRY DATE: Entered STN: 20010404  
Last Updated on STN: 20010404  
Entered Medline: 20010308  
AB The work presents a fast quantitative approach for estimating the orientations of hydrophilic and hydrophobic regions in the helical wheels of membrane-spanning alpha-helices of transmembrane proteins. The common hydropathy analysis provides an estimate of the integral hydrophobicity in a moving window which scans an amino acid sequence. The new parameter, orientation hydrophobicity, is based on the estimate of hydrophobicity of the angular segment that scans the helical wheel of a given amino acid sequence. The corresponding procedure involves the treatment of transmembrane helices as cylinders with equal surface elements for each amino acid residue. The orientation hydrophobicity, P(phi), phi = 0-360 degrees, of a helical cylinder is given as a sum of hydrophobicities of individual amino acids which are taken as the S-shaped functions of the angle between the centre of amino acid surface element and the centre of the segment. Non-zero contribution to P(phi) comes only from the amino acids belonging to the angular segment for a given angle phi. The size of the angular segment is related to the size of the channel pore. The amplitudes of amino acid S-functions are calibrated in the way that their maximum values (reached when the amino acid is completely exposed into the pore) are equal to the corresponding hydropathy index in the selected scale (here taken as Goldman-Engelman-Steitz hydropathy scale). The given

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procedure is applied in the studies of three ionic channels with well characterized **three-dimensional structures** where the channel pore is formed by a bundle of alpha-helices: cholera toxin B, nicotinic acetylcholine homopentameric alpha7 receptor, and **phospholamban**. The estimated maximum of hydrophilic properties at the helical wheels are in a good agreement with the spatial orientations of alpha-helices in the corresponding channel pores.

L6 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4  
ACCESSION NUMBER: 2000:173124 CAPLUS  
DOCUMENT NUMBER: 132:331634  
TITLE: The fast method for quantitative estimation of distribution of hydrophobic and hydrophilic segments in .alpha.-helices of membrane proteins  
AUTHOR(S): Luzhkov, V. B.; Surkov, N. F.  
CORPORATE SOURCE: Institute of Chemical Physics Problems in Chernogolovka, Russian Academy of Sciences, Chernogolovka, 142432, Russia  
SOURCE: Biol. Membr. (2000), 17(1), 74-79  
CODEN: BIMEE9; ISSN: 0233-4755  
PUBLISHER: Nauka  
DOCUMENT TYPE: Journal  
LANGUAGE: Russian

AB The work presents a fast quant. approach for estg. orientations of hydrophilic and hydrophobic regions in the helical wheels of membrane proteins. The common hydropathy anal. provides the est. of the integral hydrophobicity in a moving window that scans amino acid sequence. The

new parameter, orientation hydrophobicity, is based on the est. of hydrophobicity of the angular segment that scans the helical wheel of a given amino acid sequence. The corresponding procedure involves the treatment of transmembrane helices as cylinders with equal surface elements for each amino acid residue. The oriented hydrophobicity, P

(0),  $4=0.\text{degree}.-3600$ , of the helical cylinder is given as a sum of hydrophobicities of individual amino acids that are taken as the S-functions of the angle between the center of amino acid surface element and the center of the segment. Non-zero contribution to P (0) come only from the amino acids belonging to the angular segment for the given angle 0. The size of the angular segment is related to the size of the channel pore. The amplitudes of amino acid S-functions are calibrated in the way that their max. values (reached when the amino acid is completely exposed into the pore) equal the corresponding hydropathy index in the selected scale (here taken as Goldman - Engelman - Steitz hydropathy scale). The given procedure is applied in the studies of three ion channels with a well characterized **three-dimensional structures** where the channel pore is formed by a bundle of .alpha.-helices: cholera toxin B, nicotinic acetylcholine homo pentameric .alpha.7 receptor, and **phospholamban**. The estd. max. of hydrophilic properties at the helical wheels are in a good agreement with the spatial orientations of .alpha.-helices in the corresponding channel pores.

L6 ANSWER 7 OF 20 MEDLINE DUPLICATE 5  
ACCESSION NUMBER: 1999196736 MEDLINE  
DOCUMENT NUMBER: 99196736 PubMed ID: 10096878  
TITLE: Structure of the 1-36 amino-terminal fragment of human phospholamban by nuclear magnetic resonance and modeling  
of

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AUTHOR: the phospholamban pentamer.  
Pollesello P; Annila A; Ovaska M  
CORPORATE SOURCE: Orion Corporation, Orion Pharma, Department of  
Pharmacology  
and Drug Discovery, Cardiovascular Research, FIN-02101  
Espoo, Finland.. piero.pollesello@orion.fi  
SOURCE: BIOPHYSICAL JOURNAL, (1999 Apr) 76 (4) 1784-95.  
Journal code: A5S; 0370626. ISSN: 0006-3495.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199905  
ENTRY DATE: Entered STN: 19990525  
Last Updated on STN: 19990525  
Entered Medline: 19990507

AB The structure of a 36-amino-acid-long amino-terminal fragment of  
**phospholamban** (**phospholamban**[1-36]) in aqueous solution  
containing 30% trifluoroethanol was determined by nuclear magnetic  
resonance. The peptide, which comprises the cytoplasmic domain and six  
residues of the transmembrane domain of **phospholamban**, assumes a  
conformation characterized by two alpha-helices connected by a turn. The  
residues of the turn are Ile18, Glu19, Met20, and Pro21, which are  
adjacent to the two phosphorylation sites Ser16 and Thr17. The proline is  
in a trans conformation. The helix comprising amino acids 22-36 is well  
determined (the root mean square deviation for the backbone atoms,  
calculated for a family of 18 nuclear magnetic resonance structures is  
0.57 A). Recently, two **molecular models** of the  
transmembrane domain of **phospholamban** were proposed in which a  
symmetric homopentamer is composed of a left-handed coiled coil of  
alpha-helices. The two models differ by the relative orientation of the  
helices. The model proposed by Simmerman et al. (H.K. Simmerman, Y.M.  
Kobayashi, J.M. Autry, and L.R. Jones, 1996, J. Biol. Chem.  
271:5941-5946), in which the coiled coil is stabilized by a  
leucine-isoleucine zipper, is similar to the transmembrane pentamer  
structure of the cartilage oligomeric membrane protein determined  
recently  
by x-ray (V. Malashkevich, R. Kammerer, V Efimov, T. Schulthess, and J.  
Engel, 1996, Science 274:761-765). In the model proposed by Adams et al.  
(P.D. Adams, I.T. Arkin, D.M. Engelman, and A.T. Brunger, 1995, Nature  
Struct. Biol. 2:154-162), the helices in the coiled coil have a different  
relative orientation, i.e., are rotated clockwise by approximately 50  
degrees. It was possible to overlap and connect the structure of  
**phospholamban**[1-36] derived in the present study to the two  
transmembrane pentamer models proposed. In this way two models of the  
whole **phospholamban** in its pentameric form were generated. When  
our structure was connected to the leucine-isoleucine zipper model, the  
inner side of the cytoplasmic domain of the pentamer (where the helices  
face one another) was lined by polar residues (Gln23, Gln26, and Asn30),  
whereas the five Arg25 side chains were on the outer side. On the  
contrary, when our structure was connected to the other transmembrane  
model, in the inner side of the cytoplasmic domain of the pentamer, the  
five Arg25 residues formed a highly charged cluster.

L6 ANSWER 8 OF 20 BIOSIS COPYRIGHT 2001 BIOSIS  
ACCESSION NUMBER: 1998:442635 BIOSIS  
DOCUMENT NUMBER: PREV199800442635  
TITLE: Cysteine reactivity and oligomeric structures of



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phospholamban and its mutants.  
AUTHOR(S): Karim, Christine B.; Stamm, John D.; Karim, Jawed; Jones, Larry R.; Thomas, David D. (1)  
CORPORATE SOURCE: (1) Dep. Biochemistry, Univ. Minnesota Med. Sch., Minneapolis, MN 55455 USA  
SOURCE: Biochemistry, (Sept. 1, 1998) Vol. 37, No. 35, pp. 12074-12081.  
ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
AB To test models for the pentameric structure of phospholamban (PLB) and study its structure and molecular dynamics in SDS solution, we characterized recombinant PLB and several of its mutants by (a) reactivity of cysteine residues toward DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) and a thiol-reactive spin label, (b) oligomeric state on SDS-PAGE, and (c) EPR of the spin-labeled proteins. WT-PLB has three cysteine residues (36, 41, and 46), all located in the hydrophobic C-terminal transmembrane region. In SDS at pH 7.5, exhaustive reaction with either sulfhydryl reagent resulted in essentially 2 mol of cysteine reacted/mol of WT-PLB, with only slight destabilization of the native pentameric structure. When WT-PLB was denatured in guanidine at pH 8.1, all three cysteines reacted, disrupting the pentamer, which was restored upon cleavage of the disulfide bonds with DTT. In the tetrameric mutant C41L-PLB, the two remaining cysteine residues reacted, reversibly destabilizing the tetramer. In the monomeric mutant L37A-PLB, all three cysteines reacted. The pentameric double cysteine replacement mutant C36,46A-PLB showed negligible reactivity. We conclude that Cys-41 is the unreactive cysteine in PLB and is located at a crucial site for the maintenance of the pentameric structure. EPR spectra in SDS of spin-labeled WT-PLB and mutants correlate with the oligomeric state on SDS-PAGE; oligomeric proteins show decreased spin-label mobility compared with monomers. Molecular dynamics calculations were used to construct an atomic model for the transmembrane region of the PLB pentamer, constrained by previous mutagenesis results and the results of the present study. We conclude that (a) the mobilities of spin-labels attached to PLB and its mutants are sensitive to oligomeric state and (b) the pattern of cysteine reactivity, spin-label mobility, and oligomeric state supports a structural model for the PLB pentamer in which interactions between each pair of subunits are stabilized by a leucine-isoleucine zipper.

L6 ANSWER 9 OF 20

MEDLINE

DUPLICATE 6

ACCESSION NUMBER: 1998170970 MEDLINE

DOCUMENT NUMBER: 98170970 PubMed ID: 9512019

TITLE: Using experimental information to produce a model of the transmembrane domain of the ion channel phospholamban.

AUTHOR: Herzyk P; Hubbard R E

CORPORATE SOURCE: Department of Chemistry, University of York, Heslington, England.. pavel@yorvic.york.ac.uk

SOURCE: BIOPHYSICAL JOURNAL, (1998 Mar) 74 (3) 1203-14.  
Journal code: A5S; 0370626. ISSN: 0006-3495.

PUB. COUNTRY: United States

CEP-HTM

Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199805  
ENTRY DATE: Entered STN: 19980520  
Last Updated on STN: 19980520  
Entered Medline: 19980512

AB **Molecular models** of the transmembrane domain of the **phospholamban** pentamer have been generated by a computational method that uses the experimentally measured effects of systematic single-site mutations as a guiding force in the modeling procedure. This method makes the assumptions that 1) the **phospholamban** transmembrane domain is a parallel five-helix bundle, and 2) nondisruptive mutation positions are lipid exposed, whereas 3) disruptive or partially disruptive mutations are not. Our procedure requires substantially less computer time than systematic search methods, allowing rapid assessment of the effects of different experimental results on the helix arrangement. The effectiveness of the approach is investigated in test calculations on two helix-dimer systems of known structure. Two independently derived sets of mutagenesis data were used to define the restraints for generating models of **phospholamban**. Both resulting models are left-handed, highly symmetrical pentamers. Although the overall bundle geometry is very similar in the two models, the orientation of individual helices differs by approximately 50 degrees, resulting in different sets of residues facing the pore. This demonstrates how differences in restraints can have an effect on the model structures generated, and how the violation of these restraints can identify inconsistent experimental data.

L6 ANSWER 10 OF 20 MEDLINE DUPLICATE 7  
ACCESSION NUMBER: 2000071613 MEDLINE  
DOCUMENT NUMBER: 20071613 PubMed ID: 10603946  
TITLE: Direct spectroscopic detection of molecular dynamics and interactions of the calcium pump and phospholamban.  
AUTHOR: Thomas D D; Reddy L G; Karim C B; Li M; Cornea R; Autry J M; Jones L R; Stamm J  
CORPORATE SOURCE: Department of Biochemistry, University of Minnesota Medical School, Minneapolis 55455, USA.. ddt@ddt.biochem.umn.edu  
CONTRACT NUMBER: GM27906 (NIGMS)  
HL06308 (NHLBI)  
HL49428 (NHLBI)  
SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1998 Sep 16) 853 186-94. Ref: 23  
Journal code: 5NM; 7506858. ISSN: 0077-8923.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
(REVIEW, TUTORIAL)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200001  
ENTRY DATE: Entered STN: 20000124  
Last Updated on STN: 20000124  
Entered Medline: 20000107

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AB In order to test **molecular models** of cardiac calcium transport regulation, we have used spectroscopy to probe the structures, dynamics, and interactions of the Ca pump (Ca-ATPase) and **phospholamban** (PLB) in cardiac sarcoplasmic reticulum (SR) and in reconstituted membranes. Electron paramagnetic resonance (EPR) and phosphorescence of probes bound to the Ca pump show that the activity of the pump is quite sensitive to its oligomeric interactions. In cardiac SR, PLB aggregates and inhibits the pump, and both effects are reversed by PLB phosphorylation. Previous analyses of PLB's oligomeric state were only in detergent solutions, so we used EPR and fluorescence to determine the oligomeric structure of PLB in its native state in lipid bilayers. Wild-type PLB is primarily oligomeric in the membrane, while the mutant L37A-PLB is monomeric. For both proteins, phosphorylation shifts the dynamic monomer-oligomer equilibrium toward oligomers, and induces a similar structural change, as indicated by tyrosine fluorescence; yet L37A-PLB is more effective than wild-type PLB in inhibiting and aggregating the pump. Fluorescence energy transfer shows that the Ca pump increases the fraction of monomeric PLB, indicating that the pump preferentially binds monomeric PLB. These results support a reciprocal aggregation model for Ca pump regulation, in which the Ca pump is aggregated and inhibited by association with PLB monomers, and phosphorylation of PLB reverses these effects while decreasing the concentration of PLB monomers. To investigate the structure of the PLB pentamer in more detail, we measured the reactivities of cysteine residues in the transmembrane domain of PLB, and recorded EPR spectra of spin labels attached to these sites. These results support an atomic structural model, based on molecular dynamics simulations and mutagenesis studies, in which the PLB pentamer is stabilized by a leucine-isoleucine zipper within the transmembrane domain.

L6 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:744645 CAPLUS

DOCUMENT NUMBER: 130:121022

TITLE: Models for the transmembrane region of the phospholamban pentamer: which is correct?

AUTHOR(S): Adams, Paul D.; Lee, Albert S.; Brunger, Axel T.; Engelman, Donald M.

CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, 06520, USA

SOURCE: Ann. N. Y. Acad. Sci. (1998), 853 (Cardiac Sarcoplasmic

Reticulum Function and Regulation of Contractility), 178-185

CODEN: ANYAA9; ISSN: 0077-8923

PUBLISHER: New York Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Phospholamban is a 52-amino acid protein that assembles into a pentamer in

the membranes of the sarcoplasmic reticulum. Pentamer formation is driven

in large part by interactions of the transmembrane regions of the protein,

which are thought to be arranged as interacting .alpha.-helixes. The structural properties of phospholamban have been studied by mutagenesis and optical spectroscopy, resulting in a large database. In this discussion, we present advances in computational modeling, which identifies two probable structures for the transmembrane pentamer. A new approach to mutagenesis is described, which should lead to a clear distinction between the two possible models.

REFERENCE COUNT: 16  
 REFERENCE(S): (1) Adams, P; Nat Struc Biol 1995, V2(2), P154 CAPLUS  
 (3) Arkin, I; Annu Rev Biophys Biomol Struct 1997, V26, P157 CAPLUS  
 (4) Arkin, I; EMBO J 1994, V13, P4757 CAPLUS  
 (5) Arkin, I; J Mol Biol 1995, V248(4), P824 CAPLUS  
 (6) Fujii, J; J Biol Chem 1989, V264(22), P12950 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2001 ACS  
 ACCESSION NUMBER: 1998:744637 CAPLUS  
 DOCUMENT NUMBER: 130:121250  
 TITLE: Structural studies on phospholamban and implications for regulation of the Ca<sup>2+</sup>-ATPase  
 AUTHOR(S): Mortishire-Smith, Russell J.; Broughton, Howard; Garsky, Victor M.; Mayer, Ernest J.; Johnson, Robert G. , Jr.  
 CORPORATE SOURCE: Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, CM20 2QR, UK  
 SOURCE: Ann. N. Y. Acad. Sci. (1998), 853(Cardiac Sarcoplasmic Reticulum Function and Regulation of Contractility), 63-78  
 CODEN: ANYAA9; ISSN: 0077-8923  
 PUBLISHER: New York Academy of Sciences  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB The cardiac sarcoplasmic reticulum (SR) protein phospholamban (PLB) is an endogenous inhibitor of the SR Ca<sup>2+</sup>-ATPase. Phosphorylation of PLB relieves this inhibition and up-regulates calcium transport. PLB has proved remarkably difficult to study by conventional soln.-state NMR methods, due primarily to the extreme hydrophobic nature of the protein and its propensity to form pentamers. That the C-terminal domain of PLB is helical and membrane spanning is now well established; the structure of the cytoplasmic domain is relatively ill defined. In order to discern the effect of phosphorylation on the structure of the cytoplasmic domain, we have characterized a variety of model peptides in several structure-inducing and/or lipid-mimicking environments using CD and soln.-state NMR. The resoln. of peptide structures obtained in aq. trifluoroethanol was markedly improved by the incorporation of <sup>15</sup>N labels into the peptide backbone, allowing a variety of isotope edited, filtered, and resolved techniques to be applied. Mol. dynamics simulations on the full-length protein were combined with an anal. of published data to suggest a revised model for the structure of PLB.  
 REFERENCE COUNT: 37

CEP-HTM

REFERENCE(S): (1) Arkin, I; J Mol Biol 1995, V248(4), P824 CAPLUS  
(2) Arkin, I; J Mol Biol 1995, V248(4), P824 CAPLUS  
(3) Bax, A; J Magn Reson 1983, V55(2), P301 CAPLUS  
(4) Bodenhausen, G; J Magn Reson 1980, V37(1), P93  
CAPLUS  
(5) Cornea, R; Biochemistry 1997, V36(10), P2960  
CAPLUS  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 13 OF 20 MEDLINE DUPLICATE 8  
ACCESSION NUMBER: 97205751 MEDLINE  
DOCUMENT NUMBER: 97205751 PubMed ID: 9138559  
TITLE: A novel method for structure-based prediction of ion  
channel conductance properties.  
AUTHOR: Smart O S; Breed J; Smith G R; Sansom M S  
CORPORATE SOURCE: Department of Crystallography, Birkbeck College,  
University of London, England. o.smart@mail.cryst.bbk.ac.uk; www:  
http://www.cryst.bbk.ac.uk/-ubcg8ab/smart.html.  
SOURCE: BIOPHYSICAL JOURNAL, (1997 Mar) 72 (3) 1109-26.  
Journal code: A5S; 0370626. ISSN: 0006-3495.  
PUB. COUNTRY: United States  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199705  
ENTRY DATE: Entered STN: 19970514  
Last Updated on STN: 19970514  
Entered Medline: 19970505  
AB A rapid and easy-to-use method of predicting the conductance of an ion  
channel from its **three-dimensional structure**  
is presented. The method combines the pore dimensions of the channel as  
measured in the HOLE program with an Ohmic model of conductance. An  
empirically based correction factor is then applied. The method yielded  
good results for six experimental channel structures (none of which were  
included in the training set) with predictions accurate to within an  
average factor of 1.62 to the true values. The predictive r2 was equal to  
0.90, which is indicative of a good predictive ability. The procedure is  
used to validate model structures of alamethicin and **phospholamban**  
. Two genuine predictions for the conductance of channels with known  
structure but without reported conductances are given. A modification of  
the procedure that calculates the expected results for the effect of the  
addition of nonelectrolyte polymers on conductance is set out. Results  
for a cholera toxin B-subunit crystal structure agree well with the measured  
values. The difficulty in interpreting such studies is discussed, with  
the conclusion that measurements on channels of known structure are required.  
L6 ANSWER 14 OF 20 BIOSIS COPYRIGHT 2001 BIOSIS  
ACCESSION NUMBER: 1996:442066 BIOSIS  
DOCUMENT NUMBER: PREV199699164422  
TITLE: Helix-helix interactions in membranes: A new target for  
drugs.  
AUTHOR(S): Engelman, Donald M.  
CORPORATE SOURCE: Dep. Molecular Biophysics and Biochem., Yale Univ., New  
Haven, CT 06520 USA  
SOURCE: Schwartz, T. W. [Editor]; Hjorth, S. A. [Editor]; Kastrup,

CEP-HTM

J. S. [Editor]. Alfred Benzon Symposium, (1996) Vol. 39, pp. 122-137. Alfred Benzon Symposium; Structure and function of 7TM receptors.  
Publisher: Munksgaard 35 Norre Sogade, DK 1370 Copenhagen, Denmark.  
Meeting Info.: Meeting Hellerup, Denmark June 11-15, 1995  
ISSN: 0105-3639. ISBN: 87-16-11603-8.

DOCUMENT TYPE: Book; Conference  
LANGUAGE: English

L6 ANSWER 15 OF 20 MEDLINE DUPLICATE 9  
ACCESSION NUMBER: 95271668 MEDLINE  
DOCUMENT NUMBER: 95271668 PubMed ID: 7752243  
TITLE: Structural model of the phospholamban ion channel complex in phospholipid membranes.  
AUTHOR: Arkin I T; Rothman M; Ludlam C F; Aimoto S; Engelman D M; Rothschild K J; Smith S O  
CORPORATE SOURCE: Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, USA.  
CONTRACT NUMBER: GM 22778 (NIGMS)  
GM 46732 (NIGMS)  
GM 47527 (NIGMS)  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 May 12) 248 (4) 824-34.  
Journal code: J6V; 2985088R. ISSN: 0022-2836.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199506  
ENTRY DATE: Entered STN: 19950629  
Last Updated on STN: 19950629  
Entered Medline: 19950620

AB **Phospholamban** is a 52 amino acid residue membrane protein involved with the regulation of calcium levels across sarcoplasmic reticulum membranes in cardiac muscle cells. The N-terminal 30 amino acid residues of the protein are largely hydrophilic and include two sites whose phosphorylation is thought to dissociate an inhibitory complex between **phospholamban** and  $\text{Ca}^{2+}$  ATPase. The C-terminal 22 amino acid residues are largely hydrophobic, anchor the protein in the membrane and are responsible for  $\text{Ca}^{2+}$  selective ion conductance. Specific interactions between the transmembrane domains stabilize a pentameric protein complex. We have obtained circular dichroism (CD), transmission Fourier transform infrared (FTIR) and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra of the full-length protein and have compared these results to those from a 28 residue peptide that includes the transmembrane domain. Both proteins reconstituted into phospholipid membranes are largely alpha-helical by CD and FTIR. Polarized ATR-FTIR measurements show that both the cytosolic and transmembrane helices are oriented perpendicular to the membrane plane with a tilt of 28 (+/- 6) degrees with respect to the membrane normal. This tilt angle is in close agreement to that calculated from a model for the transmembrane domain of **phospholamban** suggested by mutagenesis and **molecular modeling**. Phosphorylation does not significantly change the secondary structure or orientation of the protein. The pentameric complex is modeled as a left-handed coiled-coil of five long helices (40 (+/- 3) residues) that extend across the membrane from the luminal carboxy terminus to the phosphorylation site in the cytoplasm. The helix bundle

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forms a perpendicular ion pore that may begin at a distance (17 to 29 Å) from the membrane surface. Based on the above, we propose a mechanism by which **phospholamban** regulates Ca<sup>2+</sup> levels across membranes that takes into account both its selective ion conductance and inhibitory association with the Ca<sup>2+</sup> pump.

L6 ANSWER 16 OF 20 MEDLINE DUPLICATE 10  
ACCESSION NUMBER: 95269058 MEDLINE  
DOCUMENT NUMBER: 95269058 PubMed ID: 7749920  
TITLE: Computational searching and mutagenesis suggest a structure  
COMMENT: for the pentameric transmembrane domain of phospholamban.  
Comment in: Nat Struct Biol. 1995 Feb;2(2):83-4  
AUTHOR: Adams P D; Arkin I T; Engelman D M; Brunger A T  
CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.  
SOURCE: NATURE STRUCTURAL BIOLOGY, (1995 Feb) 2 (2) 154-62.  
Journal code: B98; 9421566. ISSN: 1072-8368.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199506  
ENTRY DATE: Entered STN: 19950629  
Last Updated on STN: 19950629  
Entered Medline: 19950622  
AB Structural and environmental constraints greatly simplify the folding problem for membrane proteins. Computational methods can be used in a global search to find a small number of chemically reasonable models within these constraints, such that a modest set of experimental data can distinguish among them. We show that, for **phospholamban**, the global search can be further simplified by reducing the problem to two-body, rather than many-body, interactions. This method of a constrained global search combined with experimental mutagenesis data yields a **three-dimensional structure** for this pentameric ion channel. The model is a left-handed symmetric homopentamer of alpha-helices with a well-defined channel, lined solely by hydrophobic residues.

L6 ANSWER 17 OF 20 MEDLINE DUPLICATE 11  
ACCESSION NUMBER: 95045366 MEDLINE  
DOCUMENT NUMBER: 95045366 PubMed ID: 7525269  
TITLE: Structural organization of the pentameric transmembrane alpha-helices of phospholamban, a cardiac ion channel.  
AUTHOR: Arkin I T; Adams P D; MacKenzie K R; Lemmon M A; Brunger A T; Engelman D M  
CORPORATE SOURCE: Department of Cell Biology, Yale University School of Medicine, New Haven, CT.  
CONTRACT NUMBER: 5PO1-GM39546 (NIGMS)  
SOURCE: EMBO JOURNAL, (1994 Oct 17) 13 (20) 4757-64.  
Journal code: EMB; 8208664. ISSN: 0261-4189.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199412  
ENTRY DATE: Entered STN: 19950110

Last Updated on STN: 19960129

Entered Medline: 19941201

AB **Phospholamban** is a 52 amino acid calcium regulatory protein found as pentamers in cardiac SR membranes. The pentamers form through interactions between its transmembrane domains, and are stable in SDS. We have employed a saturation mutagenesis approach to study the detailed interactions between the transmembrane segments, using a chimeric protein construct in which staphylococcal nuclease (a monomeric soluble protein) is fused to the N-terminus of **phospholamban**. The chimera forms pentamers observable in SDS-PAGE, allowing the effects of mutations upon the oligomeric association to be determined by electrophoresis. The disruptive effects of amino acid substitutions in the transmembrane

domain

were classified as sensitive, moderately sensitive or insensitive. Residues of the same class lined up on faces of a 3.5 amino acids/turn helical projection, allowing the construction of a model of the interacting surfaces in which the helices are associated in a left-handed pentameric coiled-coil configuration. **Molecular modeling** simulations (to be described elsewhere in detail) confirm that the

helices

readily form a left-handed coiled-coil helical bundle and have yielded **molecular models** for the interacting surfaces, the best of which is identical to that predicted by the mutagenesis. Residues lining the pore show considerable structural sensitivity to mutation, indicating that care must be taken in interpreting the results of mutagenesis studies of channels. The cylindrical ion pore (minimal diameter of 2 Å) appears to be defined largely by hydrophobic residues (I40, L43 and I47) with only two mildly polar elements contributed by sulfurs in residues C36 and M50.

L6 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1990:473066 CAPLUS

DOCUMENT NUMBER: 113:73066

TITLE: Molecular structure and function of phospholamban: the regulatory protein of calcium pump in cardiac sarcoplasmic reticulum

AUTHOR(S): Tada, Michihiko; Kadoma, Masaaki; Fujii, Junichi; Kimura, Yoshihiro; Kijima, Yoshiyuki

CORPORATE SOURCE: Sch. Med., Osaka Univ., Osaka, 553, Japan

SOURCE: Adv. Exp. Med. Biol. (1989), 255(Calcium Protein Signaling), 79-89

CODEN: AEMBAP; ISSN: 0065-2598

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 35 refs., of the functional and structural characteristics of the **phospholamban**-ATPase system. A **mol. model** for the functional unit of **phospholamban**, which provides a basic understanding for the regulatory mechanism of ion transport and bioenergetic transduction across biomembrane, is discussed.

L6 ANSWER 19 OF 20 MEDLINE

ACCESSION NUMBER: 89313452 MEDLINE

DOCUMENT NUMBER: 89313452 PubMed ID: 2855362

TITLE: Regulation of cardiac sarcoplasmic reticulum function by phospholamban.

AUTHOR: Edes I; Kranias E G

CORPORATE SOURCE: Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, OH 45267-0575.



CEP-HTM

CONTRACT NUMBER: HL22619 (NHLBI)  
HL26057 (NHLBI)  
SOURCE: MEMBRANE BIOCHEMISTRY, (1987-88) 7 (3) 175-92. Ref: 90  
Journal code: MV5; 7804153. ISSN: 0149-046X.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
(REVIEW, ACADEMIC)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198908  
ENTRY DATE: Entered STN: 19900309  
Last Updated on STN: 19970203  
Entered Medline: 19890825

AB Calcium fluxes across the sarcoplasmic reticulum membrane are regulated by

phosphorylation of a 27,000-dalton membrane-bound protein termed **phospholamban**. **Phospholamban** is phosphorylated by three different protein kinases (cAMP-dependent, Ca<sup>2+</sup>-CAM-dependent and Ca<sup>2+</sup>-phospholipid dependent) at apparently distinct sites.

Phosphorylation

by each of the protein kinases increases the rates of active calcium transport by sarcoplasmic reticulum vesicles. The stimulatory effects of protein kinases on the calcium pump may be reversed by an endogenous protein phosphatase activity. The phosphoprotein phosphatase can dephosphorylate both the cAMP-dependent and the Ca<sup>2+</sup>-CAM-dependent sites of **phospholamban**. Phosphorylation of **phospholamban** also occurs in situ, in perfused beating hearts, during the peak of the inotropic response to beta-adrenergic stimulation. Reversal of the stimulatory effects is associated with dephosphorylation of **phospholamban**. Thus, in vivo and in vitro studies suggest that **phospholamban** is a regulator for the calcium pump in cardiac sarcoplasmic reticulum. The degree of **phospholamban** phosphorylation determined by the interaction of specific protein kinases and phosphatases may represent an important control for sarcoplasmic reticulum function and, thus, for the contraction-relaxation cycle in the myocardium. In this review, we summarize recent evidence on physical and structural properties of **phospholamban**, the proposed structural **molecular models** for this protein, and the significance of its regulatory role both in vitro and in situ.

L6 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1989:589630 CAPLUS  
DOCUMENT NUMBER: 111:189630  
TITLE: Molecular structure and function of phospholamban, the  
regulatory protein of calcium pump in cardiac sarcoplasmic reticulum  
AUTHOR(S): Tada, Michihiko; Kadoma, Masaaki; Kimura, Yoshihiro; Kijima, Yoshiyuki  
CORPORATE SOURCE: Sch. Med., Osaka Univ., Osaka, 553, Japan  
SOURCE: Calcium Signal Cell Response (1988), 3-15.  
Editor(s): Yagi, Koichi; Miyazaki, Tamotsu. Jpn. Sci. Soc. Press: Tokyo, Japan.  
CODEN: 56QPAS  
DOCUMENT TYPE: Conference; General Review  
LANGUAGE: English

CEP-HTM

AB A review, with 34 refs., on **phospholamban** (PL) with emphasis on the stimulatory action of PL on Ca pump ATPase, mol. characteristics of purified PL, **mol. model** of PL-ATPase system, and physiol. relevance of the PL-ATPase system.

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(FILE 'HOME' ENTERED AT 07:35:39 ON 21 DEC 2001)

FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 07:35:58 ON 21 DEC 2001

L1 3823 S PHOSPHOLAMBAN  
L2 15 S L1 (P) (THREE DIMENSIONAL STRUCTURE)  
L3 0 S L1 (P) (X RAY STRUCTURE)  
L4 31 S L1 (P) (MOLECULAR MODEL?)  
L5 44 S L2 OR L4  
L6 20 DUPLICATE REMOVE L5 (24 DUPLICATES REMOVED)

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